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Epstein-Barr virus associated with primary CNS lymphoma and disseminated BCG infection in a child with AIDS

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Disseminated BCG infection;
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Summary

Background: AIDS patients are at increased risk of developing concurrent infections with viral, parasitic, fungal or mycobacterial organisms. They can present constitutional symptoms of fever and weight loss, either due to infections or an underlying lymphoma which may coexist.
Case report: A child with HIV-AIDS and mild encephalopathy is reported, who during the course of a confirmed disseminated mycobacterial disease developed neurological impairment. Post-mortem examination revealed disseminated BCG infection and Epstein-Barr associated primary CNS lymphoma. Epstein-Barr virus (EBV) presence was assessed by LMP-1 protein labelling by immunohistochemistry and in situ hybridisation (ISH) for Epstein-Barr virus-encoded RNAs (EBERs) in formalin-fixed and paraffin-embedded sections.
Conclusions: BCG vaccination among HIV-1 infected children leads to the risk of disseminated BCG infection. BCG immunization programmes should be reconsidered for children at risk of HIV infection, because the risk of delayed complications is independent of the immunological status at the time of the vaccination.

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Only isolated cases of primary CNS lymphoma occurring in HIV-infected children have been reported, and a striking association with EBV infection has been demonstrated.

Introduction

Human immunodeficiency virus infection produces a broad spectrum of diseases in both children and adults. AIDS patients are at increased risk of developing opportunistic infections. Patients may present symptoms of fever and weight loss, either due to infections or a coexisting lymphoma. In children, opportunistic, reactivated latent infection and CNS lymphoma have been infrequent according to neuroradiological and autopsy examination. The most frequent CNS complication is progressive encephalopathy attributable to primary HIV infection of the brain.[1]

Primary CNS lymphoma represents a frequent complication of HIV infection in adults, occurring in as many as 6—10% of patients with AIDS, however, only isolated cases of primary CNS lymphoma occurring in HIV-infected children have been reported,[2—5] and a striking association with Epstein-Barr virus (EBV) infection has been demonstrated.[5—7]

Disseminated infection by bacillus Calmette-Guérin (BCG) is a rare but severe complication of BCG vaccination in immunodeficient patients. There is an increasing number of reports on this topic. Local or disseminated BCG disease has historically been a disease of infants, but cases in adults and older children with AIDS have been reported.[8,9]

A case is reported here of a child with AIDS with a mild HIV encephalopathy, who during the course of confirmed disseminated mycobacterial disease developed neurological impairment. Post-mortem examination revealed disseminated BCG infection and Epstein-Barr virus associated primary CNS lymphoma.

Material and methods

Immunohistochemistry (IHC)

The detection of latent membrane protein-1 (LMP-1) of EBV was performed using a pool of four monoclonal antibodies (MoAbs) CS1-4 (Dako Corporation, Carpinteria, CA, USA). Immunohistochemical characterization of the lymphoma was performed using antibodies to CD20 (L26), CD3 (PS1) (BioGenex, San Ramon, CA, USA) CD30 (Ber-H2), CD15 (Leu-M1) and CD68 (PG-M1) (Dako Corporation, Carpinteria, CA, USA). Immunohistochemical detection on formalin fixed paraffin embedded tissues employed the streptavidin-biotin complex with horseradish peroxidase technique according to the manufacturer's instructions (Dako Corporation, CA, USA).

In situ hybridization (ISH)

Fluorescein isothiocyanate (FITC)-conjugated Epstein-Barr encoded RNA (EBERs) oligonucleotides were used as probes for the hybridization procedure on paraffin sections. EBERs ISH was performed as previously described by Preciado et al.[10] Detection of the hybridized sites was achieved using a monoclonal antibody anti-FITC labelled with alkaline phosphatase (Novocastra, Newcastle-upon-Tyne, UK), performed according to the manufacturer's instructions.

Polymerase chain reaction assay for *Mycobacterium tuberculosis*

Preparation of DNA: Samples submitted were sputum, CSF and blood. Sputum was decontaminated and digested by the NALC-NaOH method.[11] CSF was concentrated by centrifugation. Both samples were then treated with proteinase K. Peripheral blood mononuclear cells were separated and DNA was obtained.

PCR: Amplification of DNA was designed as nested-PCR using the oligonucleotides described by Eisenach and Nolte as primers IS-1 (884—865), IS-2 (568—588) and IS-3 (762—781).[12,13] Primer IS-4 (910—891) was personally selected from the complete sequence published by Eisenach. The amplified region (123 bp) belongs to the IS6110 repeated sequence which is specific for *Mycobacterium tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*).[11,12] Both PCRs were performed in a final volume of 50 μl containing 1.5 mM $MgCl_2$; 200 μM (each) dNTPs; 0.5 μM (each) primers; 1.25 U of *Taq* DNA polymerase; 1× PCR buffer provided with the enzyme, and 5 μl of DNA or 20 μl of lysis supernatant. The reaction was made in a touchdown system from 70 °C to 68 °C for the first round (IS-2-IS-4) and 74 °C to 68 °C for the second (IS-1-IS-3). Inhibition was also evaluated.

The following biochemical characteristics were used to identify *M. bovis*-BCG: negative niacin test;